

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

CHANGLING LIU, *et al.*

Serial No.: 10/598,033

Filed: 16 August 2006

For: SUBSTITUTED AZOLE COMPOUNDS AND ITS  
PREPARATION AND USE THEREOF

Confirmation No. 9375

Art Unit: 1617

Examiner: Zarek, Paul E.

Atty. Dckt: 034266 M 003

**RULE 1.132 DECLARATION  
BY CHANGLING LIU**

1. I, Changling Liu, a resident of Shenyang, China, declare the following:
2. I am a listed inventor on the above-referenced application.
3. My curriculum vitae is attached as Exhibit A.
4. I have reviewed and understand the above-referenced application, i.e. the specification and the currently pending claims (Claimed Invention).
5. I have reviewed and understand Desbordes (WO 99/33812) and the Office Action dated 14 October 2009.
6. My co-inventors and I had Dow AgroSciences Ltd. (Dow) conduct experiments to evidence the unexpected and superior results of the Claimed Invention. Our request to Dow and the results Dow returned are attached as Exhibit B.
7. It is my opinion that a person skilled in the art would find that compound 2 as set forth in the above-referenced application is representative of the compounds of the Claimed Invention because of their structural similarities.
8. It is my understanding that the experimental protocol for testing the fungicidal activities as carried out by Dow was the standardized 1 day protectant test (1DP) as set forth in pages 53-56 of WO2006047397A1 as follows:

Leaf Rust of Wheat (causal agent *Puccinia recondita tritici* = *Puccinia triticina*; Bayer code PUECRT): Wheat plants (variety Yuma) were grown from seed in a soil-less peat-based potting mixture (Metromix) until the seedlings had a fully expanded first leaf. Each pot contained 3-8 seedlings. These plants were sprayed until wet with the formulated test compounds. On the following day, the leaves were inoculated with an aqueous spore suspension of *Puccinia recondita tritici* and the plants were kept in high humidity overnight to permit spores to germinate and infect the leaf. The plants were then transferred to a greenhouse until disease developed on untreated control plants.

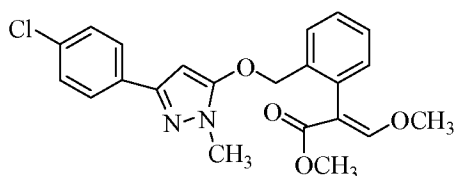
### Biological Testing

The activity of the compounds as effective fungicides was determined by applying the compounds to plants and observing control of fungal disease. The compounds were formulated at 200 ppm in 10 vol. percent acetone plus 90 vol. percent Triton X water (deionized water 99.99 wt. percent + 0.01 wt. percent Triton X100), giving a “formulated test compound.” In a few cases, compounds were formulated at 100, 75 or 8.3 ppm rather than 200 ppm in 10 vol. percent acetone plus 90 vol. percent Triton X water (deionized water 99.99 wt. percent + 0.01 wt. percent Triton X100), giving a ‘formulated test compound’. The compounds were tested for ability to control plant diseases in a 1-day protectant test (1DP) or a 2-day curative test (2DC). Formulated test compounds were applied to plants using a turn table sprayer fitted with two opposing air atomization nozzles which delivered approximately 1500 L/ha of spray volume. Plants were inoculated with spores of the fungus the next day (1DP), then incubated in an environment conducive to disease development. In a few cases, the compounds were tested for ability to control plant disease in a two-day curative test. Plants were inoculated with spores of the fungus two days prior to compound application, and incubated in an environment conducive to disease development both before and after compound application (2DC). For all types of tests, disease severity was evaluated 4 to 28 days later, depending on the speed of disease development.

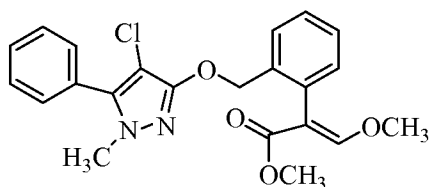
9. As set forth in Exhibit B, XS8-SYP3343 is compound 2 and PUCCRT = *Puccinia recondita*. The results set forth in Exhibit B evidence that compound 2 is 100% effective at 12.5 ppm against *Puccinia recondita*.
10. Although side-by-side experiments were not conducted by Dow with compound 73 of Desbordes, it is noted that Example B1 of Desbordes does not list compound 73. Thus, at 40 ppm, compound 73 was not at least 75% effective against *Puccinia recondita*.
11. I was surprised that the Dow data indicates that compound 2 of the present invention is 100% effective at only 12.5 ppm against *Puccinia recondita*. Thus, compound 2 is 100% effective at 1/3 the dose as compared to compound 73 of Desbordes. Thus, it can be said that compound 2 is at least 66% more active against *Puccinia recondita* as compared to compound 73 of Desbordes.
12. In my opinion, a person skilled in the art would find that the antifungal activities of compound 2 as provided by Dow, to be superior and unexpected in view of that disclosed in Desbordes.

13. It is also my opinion, that a person of ordinary skill in the art would not have a reasonable expectation that position 5 isomers of the compounds disclosed in Desbordes, including compound 73, would result in an improvement in antifungal activity by at least 66%.
14. My co-inventors and I conducted additional experiments between compound 2 and SYP-3342 to evidence the unexpected and superior results of the Claimed Invention.
15. The structural formulas of compound 2, SYP-3342 and compound 73 of Desbordes are as follows:

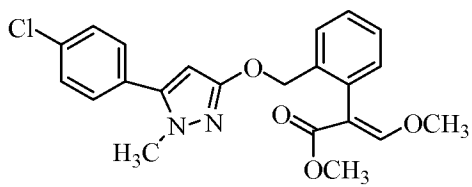
The formula of compound 2 (SYP-3343) in the invention is as follows:



The formula of compound 73 of Desbordes is as follows:



The formula of SYP-3342 is as follows:



16. It is my opinion that a person skilled in the art would find that SYP-3342 is representative of compound 73 of Desbordes because of their structural similarities.
17. My co-inventors and I determined the 1DP antifungal activities of compound 2 and SYP-3342 as follows: The compounds were tested for ability to control plant diseases in a 1-day protectant test (1DP). Technical samples were dissolved in a bit acetone and diluted to required concentration with water containing 0.1% Tween 80. Test solution was sprayed onto potted plant. Pathogen inoculation was carried out after 24 hours then plants were hold in growth chambers containing constant temperature and moisture for effect. When untreated plant was under desirable disease severity (after about 1 week), assessment were carried out by visual observation.
18. The activities provided below are the average of two repeated tests according to the protocol in #17 above.
  - a. The 1DP activities of compound 2 and SYP-3342 against wheat powdery mildew (*E.*

*graminis*) after 7 days are set forth in Table B.

| Table B                  |                         |        |          |          |          |
|--------------------------|-------------------------|--------|----------|----------|----------|
| compound                 | Activity after 7 days % |        |          |          |          |
|                          | 200 ppm                 | 50 ppm | 12.5 ppm | 3.13 ppm | 0.78 ppm |
| SYP-3342                 | 100                     | 50     | 0        | 0        | 0        |
| compound 2<br>(SYP-3343) | 100                     | 96.5   | 82.5     | 50       | 15       |

As shown in Table B, at 50 ppm, compound 2 exhibited almost 50% more activity than SYP-3342 after 7 days. Thus, it can be extrapolated that compound 2 is almost 50% more active than compound 73 of Desbordes against wheat powdery mildew.

- The 1DP activities of compound 2 and SYP-3342 against cucumber downy mildew after 1 day are set forth in Table C.

| Table C                  |            |          |
|--------------------------|------------|----------|
| compound                 | Activity % |          |
|                          | 25 ppm     | 6.25 ppm |
| SYP-3342                 | 0          | 0        |
| compound 2<br>(SYP-3343) | 100        | 100      |

As provided in Table C, compound 2 exhibits 100% activity against cucumber downy mildew at 6.25 and 25 ppm, whereas SYP-3342 does not. Thus, it can be extrapolated that compound 2 is 100% more active than compound 73 of Desbordes against cucumber downy mildew.

19. Assuming that the data of Desbordes indicates that compound 73 exhibits, at 40 ppm, just a little less than 75% activity against *Septoria nodorum* and 75% activity against *Puccinia recondita*, it is my opinion that the compounds of the present invention exhibit superior and unexpected antifungal activities. In particular, it is my opinion that an 18% to 25% increase in antifungal activity at 1/3 the dose is evidence the superior and unexpected properties of the Claimed Invention.
  - Specifically, the data by Dow shows that compound 2 exhibited 93% activity against *Septoria nodorum*, at 12.5 ppm. Thus, at 1/3 the dose, compound 2 exhibits at least about an 18% increase in antifungal activity.
  - Additionally, compound 2 exhibited 100% activity at 12.5 ppm against *Puccinia recondita*. Since 12.5 ppm is more than one third less than the amount needed for compound 73 to provide 75% activity, compound 2 is at least 25% more active at 1/3 the amount.
20. My co-inventors and I also tested the antifungal, insecticidal and acaridal activities of compound 2 against various pathogens. The protocol for the antifungal activities is set forth in #17 above and the protocol for the insecticidal and acaricidal activities is as

follows: Technical samples were dissolved in a bit acetone and diluted to required concentration with water containing 0.1% of Tween 80. The second instar larvae of armyworm and culex mosquitoes, cotton aphids and adult Carmine spider mite were used in biological test. The method was employed either spraying by airbrush or immersing (larvae of culex mosquitoes). A test solution (0.5 ml) was sprayed at the pressure of 10 psi (about 07 kg/cm<sup>2</sup>). Percent mortality was determined after two days. The results are as follows:

| Table D                  |                                    |     |     |      |     |     |      |   |     |     |      |   |     |     |      |     |     |
|--------------------------|------------------------------------|-----|-----|------|-----|-----|------|---|-----|-----|------|---|-----|-----|------|-----|-----|
| pathogen                 | rice blast<br>( <i>P. oryzae</i> ) |     |     |      |     |     |      | cucumber grey mold<br>( <i>B. cinerea</i> ) |     |     |      | tomato late blight<br>( <i>P. infestans</i> ) |     |     |      |     |     |
| Dose<br>ppm              | 25                                 | 8.3 | 2.7 | 0.92 | 0.3 | 0.1 | 0.03 | 25  | 8.3 | 2.7 | 0.92 | 25  | 8.3 | 2.7 | 0.92 | 0.3 | 0.1 |
| Compound 2<br>activity % | 100                                | 100 | 100 | 80   | 80  | 50  | 0    | 100   | 80  | 80  | 80   | 100   | 100 | 100 | 80   | 50  | 0   |

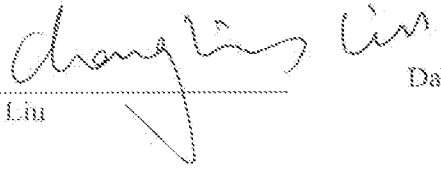
| Table E               |   |      |      |       |  |      |      |      |     |  |      |     |       |
|-----------------------|---|------|------|-------|--|------|------|------|-----|--|------|-----|-------|
| pathogen              | cucumber downy mildew<br>( <i>P. cubensis</i> ) |      |      |       | wheat powdery mildew<br>( <i>E. graminis</i> ) |      |      |      |     | cucumber anthracnose<br>( <i>C. orbiculare</i> ) |      |     |       |
| Dose ppm              | 25  | 12.5 | 6.25 | 3.125 | 25   | 6.25 | 1.56 | 0.39 | 0.1 | 25   | 12.5 | 6.3 | 3.125 |
| Compound 2 activity % | 100   | 100  | 98   | 90    | 100  | 95   | 70   | 30   | 15  | 100  | 100  | 95  | 70    |

| Table F               |                     |    |    |           |     |     |                  |     |     |              |    |      |
|-----------------------|---------------------|----|----|-----------|-----|-----|------------------|-----|-----|--------------|----|------|
| pathogen              | Carmine spider mite |    |    | army worm |     |     | culex mosquitoes |     |     | cotton aphid |    |      |
| Dose ppm              | 160                 | 80 | 40 | 400       | 200 | 100 | 20               | 10  | 5   | 50           | 25 | 12.5 |
| Compound 2 activity % | 56                  | 53 | 40 | 100       | 90  | 90  | 100              | 100 | 100 | 100          | 95 | 80   |

- As shown in Table D, compound 2 exhibited 80% activity at doses of 0.92 ppm.
  - As shown in Table E, compound 2 exhibited at least 95% activity at 6.3 ppm.
  - As shown in Table F, compound 2 exhibits insecticidal and acaridal activity.
21. In my opinion, the results set forth in #20 above evidence the unexpected and superior activities of the compounds of the Claimed Invention. It is also my opinion, that nowhere does Desbordes teach or suggest that position 5 isomers will exhibit 80% activity against rice blast, cucumber grey mold and tomato late blight at 0.92 ppm, at least 95% activity against cucumber down mildew, wheat powdery mildew and cucumber anthracnose at 6.3 ppm, and insecticidal and acaridal activity.
22. I declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may

jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Signed:

  
Changling Liu

Date:

21/01/2010